ILFORD

TECHNICAL INFORMATION

ILFORD NUCLEAR EMULSIONS

TECHNICAL INFORMATION FOR AUTORADIOGRAPHY APPLICATIONS

1. INTRODUCTION

Nuclear research emulsions were first developed in the 1940s to meet the needs of physicists engaged in research on cosmic radiation. The range of materials was improved and extended throughout the following decade until the versatility of nuclear emulsions in the recording of charged particles and ionising radiation was recognised by workers in many other fields. In addition to the use in particle physics ILFORD nuclear emulsions are now extensively used in autoradiography - in medical and biological research, in metallurgy and in the study of chemically reactive surfaces.

ILFORD manufacture nuclear emulsions for a wide range of applications. The needs of autoradiography are particularly well met by emulsions in gel form. These are particularly intended for users who need to coat their own plates or specimens to ensure a very close contact between source and emulsion to achieve maximum definition.

Other materials not listed here have been produced from time to time to meet the special requirements of individual workers. ILFORD are always pleased to discuss such special needs and, where possible, to develop new ways of applying photographic emulsions in scientific research. Email Us.

2. THE PHOTOGRAPHIC PROCESS

2.1 FORMATION OF THE LATENT IMAGE

A photographic emulsion is essentially a dispersion of silver halide crystals in a gelatin matrix. ILFORD nuclear emulsions are fundamentally the same as general purpose photographic emulsions, but have several distinguishing features:

The silver halide crystals are very uniform in size and sensitivity.
There are very few crystals that may be developed without exposure to a charged particle (very low chemical fog).
The silver to gelatin ratio is much higher than in a conventional emulsion
When such an emulsion is exposed to ionising radiation or light, clusters of silver atoms are produced. These are known as latent image centres, as they are not visible until the emulsion is developed, when all the crystals containing a latent image centre are reduced to metallic silver.

When a silver halide crystal absorbs light or ionising radiation, it has the effect of liberating mobile electrons and electron deficient bromine atoms. Transfer of an electron from an adjacent bromine ion, which in turn creates an electron deficiency, can overcome the electron deficiency of the bromine atom. In this way, a positive hole can move through the crystal lattice. This electron deficiency may also be known as a 'positive hole'.

It is important for latent image formation that a significant proportion of electrons and positive holes are trapped separately, otherwise they could recombine and regenerate halide ions. The silver halide crystal contains free (interstitial) silver ions, which can move through the lattice. When an interstitial silver ion encounters a trapped electron, the charges are neutralised and an atom of metallic silver is formed. The single atom of silver is unstable but, while it exists, it increases the efficiency of the site as an electron trap. In this way a stable nucleus of four or more atoms of silver can be built up. The site is then known as a latent image centre, and that entire crystal may be reduced to metallic silver on development.

2.2 DEVELOPMENT

Photographic development is the process by which the latent image contained in an emulsion is made visible by the reduction of silver ions in the silver halide crystal to metallic silver.

When developing ILFORD nuclear emulsions, a developer is usually chosen which reduces those crystals containing a latent image centre completely and leaves those not containing a centre unchanged. The development time used for processing material should be sufficient for those crystals with a latent image centre to be reduced completely, but not so long that unexposed crystals are developed. In practice, a certain number of crystals will be developed even though they do not contain a latent image centre. These grains, when developed, constitute what is known as fog or background.

Developing agents may be divided into two main groups, depending on the source of silver ions for reduction. In practice, most developers give a combination of the two sorts of development.

The first group is known as physical developing agents. In physical development, silver ions are provided from the solution in the form of a soluble complex. These are deposited on the latent image centre and are reduced to metallic silver. This produces spherical particles, the precise shape of which is affected by pH.

Chemical developing agents make up the second group and are more usually chosen when processing nuclear emulsions. However, the choice between a physical developer and a chemical developer will largely depend on the grain structure required in the processed image. In chemical development, silver ions are provided from the silver halide crystal containing the latent image centre. The action of a chemical developer produces a mass of filaments bearing little resemblance to the original crystal. If silver halide solvents such as sulphite are present in a chemical developer, an opportunity exists for some physical development to occur. In this case, the filaments in the processed plate will be shorter and thicker.

Chemical development, like many other chemical reactions, is dependent on temperature. In general, development occurs more rapidly at higher temperatures - below 10°C development virtually stops. For this reason it is important to keep the processing temperature constant during development, otherwise it will not be possible to assess the correct development time.

Chemical developers are also dependent on pH, and will only maintain a given activity within a narrow pH range. In general, the less alkaline the environment, the less active the developer will be. For this reason, the use of an acid stop bath such as ILFOSTOP PRO is often recommended at the end of development. This stops development immediately so that the development time can be controlled precisely.

2.3 FIXATION

The purpose of fixation is to remove all the residual silver halide, leaving the metallic silver to form the image. If the silver halide was left in the emulsion, it would slowly go brown and degrade the image. The fixing agents most widely used are sodium or ammonium thiosulphate, which form thiosulphate complexes with the silver halide. Silver thiosulphate is soluble in water and so may be removed from the emulsion by washing.

It is important to use a fixer which has not been exhausted when processing nuclear emulsions, otherwise some silver halide will remain in the emulsion. To ensure that it is all removed a fixing time should be used which is twice the time it takes for the emulsion to clear.

After fixation, the emulsion must be washed very thoroughly. This is to remove all the silver thiosulphate complexes in the emulsion. If any do remain, they will eventually break down, forming silver sulphide which is brown and will obscure the image.

3 PRODUCT RANGE

3.1 TYPES OF EMULSION

There are currently three types of ILFORD nuclear emulsions, with a fourth under development.

ILFORD emulsion	Crystal diameter (μm)
G5	0.27
K	0.20
L4	0.11

ILFORD K emulsions are available in a range of sensitivities, which are defined by a number denoting increasing sensitivity from 0 to 5.

K0	Used in particle physics to record protons of energies up to 5MeV. Records thorium α-particles as nearly continuous tracks. Not often used in autoradiography and produced only by special order.
K2	Used in autoradiography and can be used with 3H or 125I. Autoradiographers working with specimens with high activity may prefer the lower background given by K2.
K5	Used in autoradiography with any isotope. Exposure times tend to be shorter than with K2, especially where activity levels are low.

K5 emulsion is also available in a ready-to-use diluted form as K5D.

The choice of emulsion will largely depend on the viewing system and the activity of the radioactive isotope used.

Electron microscopy:
L4 is the recommended emulsion for this application as it has the highest resolution.

Light microscopy:
The K series of emulsions are usually the most appropriate for this degree of enlargement.

3.2 ORDERING

ILFORD nuclear emulsions are supplied in brown glass bottles of 50 and 100ml. It would be unpractical and generally undesirable to maintain large stocks of nuclear emulsion, but small quantities of the emulsions G5, K2, K5 and L4 are available from stock. Delivery of large quantities of emulsion is by arrangement. For further information on the availability of other nuclear emulsion products, please contact ILFORD. Email us

3.3 COMPOSITION

ILFORD nuclear emulsion contains 0.162g Ag/g emulsion and 0.042g gelatin/g emulsion. At least 65g of emulsion are contained in a 50ml bottle and 130g in a 100ml bottle.

The product is supplied in shreds for ease of handling.

The density of the material as supplied is around 1.3 g/cm3. On drying this rises to 3.8 g/cm3 in equilibrium with air at 58% relative humidity at 20°C.

4 PRODUCT USE

4.1 STORAGE BEFORE EXPOSURE

In general, nuclear emulsions should be protected from light and local radioactivity. Sensitised material should be stored in such a way that efficient stock rotation is possible to ensure that material is in optimum condition. It may be helpful to record the date when the emulsion was received on the bottle label.

Emulsions in gel form deteriorate rapidly above 5°C and must be kept under refrigeration, without freezing. The outer wrapper should be retained during storage. After coating and drying, slides may be stored at room temperature, refrigerated or frozen. When slides are stored below 5°C and are to be used at normal room temperature, they should be allowed to warm up in their packaging for about three hours to prevent condensation forming on the surface.

Sensitised materials should not be stored near to certain chemical solutions, such as ammonia, sodium sulphide or formaldehyde; or near fumes or vapours coming from volatile substances; gases, such as sulphur dioxide or coal gas; or some industrial solvents and cleaning fluids. Materials should not be stored on new or newly painted wood. Processing chemicals should be stored as far away as possible from sensitised material.

Its basic sensitivity and the exposure it receives from cosmic radiation and local radioactive sources determine the useful life of the fresh emulsion. If correctly stored, nuclear emulsions should remain in good condition for at least two months. Nuclear emulsions may be usable for considerably longer depending on the level of background acceptable in a particular application.

4.2 HANDLING / SAFETY ADVICE

The shreds can normally be removed from the bottle with plastic tweezers. The exception to this is K5D, which may be liquid. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
Use in an adequately ventilated room.

4.3 SAFELIGHT RECOMMENDATIONS

ILFORD nuclear emulsions are sensitive to blue light. For general darkroom illumination, the ILFORD 902 (light brown) safelight filter in a darkroom lamp fitted with a 15 watt bulb, is recommended. For direct illumination, the ILFORD 904 (dark brown) safelight filter is recommended. When maximum illumination is required, a sodium lamp with the correct safelight filters may be used. The safety of this type of lamp should be checked by a practical test before use.

ILFORD nuclear emulsion should not be exposed to safelighting for any longer than necessary. If preparations are to be manipulated before processing, great care should be taken to avoid prolonged exposure to safelight.

4.4 CHECKING THE EMULSION BEFORE USE

It is possible that ILFORD nuclear emulsion may have been exposed in transit to conditions causing the shreds to melt slightly and form a solid lump. These conditions may not have affected the performance of the emulsion. This should be tested before commencing an experiment. If the level of background is acceptable and the distribution of developed grains is uniform, the emulsion is undamaged and fit to use.

5 USING NUCLEAR EMULSION FOR AUTORADIOGRAPHY

Biological autoradiography takes advantage of the fact that animals and plants cannot distinguish between stable and radioactive isotopes of the same elements in their physiological reactions. This enables the path of labelled compounds to be traced in an organism using ILFORD nuclear emulsions. This technique is invaluable in the studies of drugs, pesticides and hormones.

Autoradiography has provided a useful tool in metallurgy, such as in the investigation of the distribution of different metals within an alloy. Autoradiography may also be used in studies of frictional damage by machinery. On a smaller scale, autoradiography can be used to find out more about chemically reactive surfaces. The use of ILFORD nuclear emulsion in diffusion studies may be used to demonstrate, for example, intercrystalline boundaries in metal or the permeability of natural and synthetic membranes to different substances. The range of ILFORD nuclear emulsions enables studies to be made at all levels of magnification, from actual size to enlargements made with optical and electron microscopes.

The choice of emulsion for autoradiography will always depend on the strength of the radioactive source used, the energy of the emitted particles (usually b-particles) and the discrimination required. ILFORD have a range of emulsions specially designed to satisfy a wide range of demands - see Section 3.1.

5.1 PREPARATION OF SPECIMEN

When preparing a specimen for autoradiography, it is important to use a histological fixative. This must retain the radioactive compound in the tissue during subsequent dehydrating and embedding procedures and not affect the sensitivity of the emulsion. Wash the slides on which sections are to be mounted in acid and rinse many times in distilled water. If required, the slides may be subbed to provide a good adhesive surface between the section and the emulsion. A suitable solution, into which prepared slides may be dipped, is given below.

Gelatin	0.5g
Chrome alum (K2SO4.Cr(SO4)3.24H20)	5.0g
Distilled water to	1 Litre

Make up the solution just before use. After dipping, drain the slides and dry in a dust free atmosphere.

To avoid chemographic effects (chemicals leaching out of the section into the emulsion and affecting it independently of the nuclear decay), the slide may be dipped in celloidin solution. This procedure is not recommended when low energy isotopes such as 3H and 125I are to be detected. In general, it is better to stain biological specimens before application of the nuclear emulsion. It is important that the chosen stain does not produce chemographic effects.

5.2 COATING A SLIDE FOR VISUAL EXAMINATION OR LIGHT MICROSCOPY

Ideally, the environmental conditions in the darkroom are about 25°C and 75%RH.

It has been reported that heating the emulsion before exposure will increase its sensitivity. However, the temperature to which it is heated is critical, because after a certain point, which varies according to the emulsion, the corresponding increase in fog will overshadow any benefits of increased sensitivity.

Remove enough emulsion from the bottle for immediate requirements only. Melt in a glass or stainless steel vessel with an equal volume of deionised or distilled water in a water bath at about 40°C, stirring gently to avoid local overheating. ILFORD K5D, being already diluted needs no such additional water.
NOTE. If the whole bottle is to be used in one go, loosen the cap before placing it in the water bath.

When melting do not agitate so as to produce froth. If frothing does occur, larger air bubbles can be removed by filtering. Ideally, the emulsion should be allowed to melt undisturbed for an hour in the water bath. This has the advantage of reducing the background in the coated slides. Emulsion may be reheated after it has set, but contaminated emulsion must not be returned to the stock bottle.

For most autoradiographic work, a very thin layer of emulsion is required. This can be obtained by allowing the emulsion to drain off the glass as completely as possible. Emulsion may be applied to the plate either by dipping the slide into the emulsion or by allowing a few drops of emulsion to fall onto the slide. If using undiluted emulsion the thickness of the layer when dry will be 10-15% of its thickness at this stage. For example, 0.8ml/dm^2 will produce a layer 10µm thick when dry. Hold the slide vertically on a gauze pad for draining and place horizontally for setting and drying.

It is important to produce a uniform layer of emulsion, particularly when using an isotope such as 14C, which emits high energy b-particles which will penetrate the emulsion. If the coating is not even, particles may be stopped within the emulsion in thick areas and pass right through in thin areas, giving inconsistent results.

Allow the emulsion to set in the dark, then dry it with a gentle current of clean air. The temperature and humidity of the air current are not critical, but drying at room temperature will avoid stress marks in the emulsion. Alternatively, allow the emulsion to dry on a cold, metal plate. This will slow down drying, but increase the gelling speed. Slides may also be dried in a carbon dioxide atmosphere.

Thinner layers of emulsion can be produced by diluting the emulsion with additional water, or by the addition of glycerol, which has the effect of reducing any fog resulting from stress between the gelatin and the silver halide crystals. In certain circumstances, it may be better to dilute the emulsion with gelatin. This has the effect of decreasing signal and background. This can be an advantage when examining a specimen with very high activity. In addition, emulsions diluted with gelatin are more sensitive than undiluted ones (in relation to their dilution).

Pressure marks: Rapid drying or brushing of the plate before it is completely dry causes these.

5.3 COATING A PREPARATION FOR ELECTRON MICROSCOPY

A very thin layer of emulsion is obviously necessary for electron microscopy, but little useful information can be derived from a preparation thinner than 3µm.

There are two main methods of coating ILFORD nuclear emulsions for electron microscopy; the flat substrate method and the loop method. Nuclear emulsions in gel form should never be applied directly to specimens mounted on grids as a highly uneven surface is formed.

The flat substrate method is virtually the same as the technique described above. Coat the slides with collodion or formvar-carbon before dipping in the emulsion. Dilute the emulsion with distilled water to ensure an even, thin layer.

The loop method involves dipping a wire loop into melted emulsion that has been diluted with distilled water, to form a membrane. This is applied to a specimen mounted on a grid and left for the required exposure time.

Specimens to be coated by this technique must first be sectioned and mounted on collodion-coated grids, which preferably should be gilded. The sections should be stained and coated with a thin layer of carbon. The specimens are then mounted on small corks, with double-sided adhesive tape. The tape has a hole punched in it, which is slightly smaller than the grids. This apparatus allows the best area of any emulsion membrane to be used each time.

While the usual recommendation for handling ILFORD nuclear emulsion is about 25°C, with this technique it is easier to handle it at about 18°C. Melt the emulsion and allow it to semi-gel. Test the state of gelling at intervals; it should have occurred in 20 minutes. When the emulsion has achieved the correct state, dip platinum or Nickel/chrome wire loop into the emulsion so that the loop is completely covered. Withdraw the loop edge first. Inspect the emulsion membrane in front of a safelight for any imperfections. Allow the membrane to dry in the loop. This takes about 2-3 minutes. Choose the best area and apply it to the specimen. In some circumstances, it may be necessary to add a surfactant to the melted emulsion to ensure a regular monocrystalline layer.

Insert the coated specimens into narrow glass tubes and leave in a light-tight box for the required exposure time.

5.4 EXPOSURE

The exposure time required for autoradiographic preparations will vary according to the specific activity of the isotope employed, the amount of compound incorporated and its distribution in the material. It is therefore advisable to set up several preparations so that samples can be taken at different intervals of exposure e.g. 2 days, 1 week, 3 weeks, 3-8 months. As a rough guide for biological autoradiographs, a content of 1 microcurie per gram of tissue would require about 20 days exposure, and a preparation for electron microscopy would require about 10 times more exposure than a light microscope preparation from the same block of tissue.

5.5 STORAGE DURING EXPOSURE

If exposure times are short, which is up to two days, the storage conditions during exposure are not important. The exposures encountered in autoradiography, however, may last from several days to several months. In these circumstances, it is essential that the emulsion be kept under much the same conditions as emulsion stored before use. High humidity can cause increased fog and accelerated latent image fading, so it is particularly important to maintain low humidity conditions during the exposure period. Care should be taken to ensure there is sufficient radioactive tracer to keep exposure times reasonably short to avoid latent image loss. If latent image fading still remains a problem, it may be slowed down by exposing the preparation in an atmosphere of inert gas.

Where exposure times are particularly long, the use of a lead lined box is recommended to provide protection from background radiation.

It is extremely important to process the materials immediately after the exposure time has been completed. The latent image fading is progressively more severe as the crystal size of the silver halide decreases in the order G5, K and L4. If there has to be a delay before the emulsion can be processed, then the preparation should be stored with proper protection from radiation at a temperature between 5-10°C and at 50%RH.

6 PROCESSING FOR AUTORADIOGRAPHY

A number of processing techniques are described below. These are intended as a guide and may be modified to suit individual working conditions and experimental aims. During the course of an experimental programme, processing techniques should be standardised.

In order to avoid local distortion of the emulsion layer it is important to avoid large changes in temperature, particularly during wet stages of the process. Sudden temperature swings of around 10°C can cause a defect known as reticulation. On a macroscopic level this causes a roughening of the emulsion surface similar to orange peel. On a microscopic scale there are large local changes to the fog level due to stresses and strains within the emulsion.

6.1 DEVELOPMENT

The thin layers of emulsion usually found in autoradiography may be developed in any high-energy developer.

Autoradiographs for light microscopy:
The best results will be obtained using ILFORD PHENISOL diluted 1+4 for 4 minutes at 18°C. Alternatively use ID-19 (Section 8) or Kodak D-19 diluted 1+1: use a development times of about 4 minutes at 20°C.

The development time to be used is the shortest time that will achieve complete development of all the latent image centres (see Section 2.1). Always ensure the developer is fresh and has not started to turn brown.

Reduction of signal-to-noise ratios may be achieved by further diluting a standard developer and increasing the development time. This is particularly useful when estimating the number of silver grains by reflectance.

Autoradiographs for electron microscopy:
Develop autoradiographs for electron microscopy in ID-19 (Section 8) or Kodak D-19 (undiluted) for about 2 minutes at 20°C.

If an extra-fine grain developer is required for electron microscope work, use an ascorbic acid-metol developer or the concentric Phenidone developer. These are physical developers and give a different shaped grain structure after processing than that given by a standard developer. Further details of these can be found in the autoradiography references in Section 9.

6.2 STOP BATH

After development, transfer the material to an acid stop bath. This may be made up with ILFORD ILFOSTOP PRO diluted 1+19 with water or 0.2-2% acetic acid solution.

Just rinse autoradiographs for electron microscopy in distilled water.

6.3 FIXING

The emulsion should then be fixed. Use any standard non-hardening fixer, e.g. ILFORD HYPAM diluted 1+4 for 4 minutes at room temperature. Hardening fixers are not recommended because of the difficulty of ensuring an efficient wash.

The fixing time is a function of layer thickness. Fix the material for twice the time it takes the emulsion to clear.

6.4 WASHING

After fixing, emulsions may be exposed to normal room light. The required washing time will depend on the thickness of the emulsion: about 10 minutes is needed for layers of up to 10µm. It is recommended that the wash water temperature be about the same as the temperature of the processing solutions.

Slides may be washed in tap water, but give a final rinse in distilled water. This will ensure that any materials dissolved in the tap water are not concentrated in the emulsion on drying. Failing to do this is a common cause for a lack of permanence in the final image.

6.5 STAINING

Tissue sections may be stained through the emulsion after all the residual fixer solution has been removed from the emulsion. The post-processing stain must not colour the gelatin of the emulsion. The stain should not contrast with nor obscure the developed silver grains of the emulsion. It is important that the staining solution should not remove developed silver grains from the emulsion and that the stain should not fade.

For studying autoradiographs using the light microscope, Toluidine Blue is a suitable stain. For studies using the electron microscope, the most widely used stains are alkaline lead stains. These, however, are said to cause partial removal of the gelatin. Some workers have intentionally removed the gelatin before staining to give clearer results.

However the use of stains is implicated in the lack of permanence of the silver image noted by some users. This is because some of the chemicals used have the ability under certain circumstances to bleach silver grains. In practice this means that a silver autoradiographic image visible after processing disappears over time leaving only a yellow stain. Stains such as Toluidine Blue, Methylene Blue and Neutral Red have been known to exhibit this effect. See the work of Bogoroch in Section 9.

The issue appears to be the batch to batch variation in these dyes. We suggest you screen a new batch of stain for this effect before using it on a critical set of samples.

7 STORAGE OF PROCESSED MATERIALS

After nuclear emulsions have been processed, the storage conditions are less critical than before. If plates and slides are properly processed, with adequate fixing and washing, and are correctly stored they will keep in good condition for many years. Store processed material that is to be kept for a long time at about 10°C and 50%RH.

Variation in temperature and humidity should be kept to a minimum. Store preparations in the dark while they are not being examined.

8 DEVELOPER FORMULATIONS

ID-19

Metol	2.2g
Sodium sulphite, anhydrous	72g
Hydroquinone	8.8g
Sodium carbonate, anhydrous	48g
Potassium bromide	4g
Water to	1 litre

9 USEFUL REFERENCES

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